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(57) Abstract

Methods of treating or preventing fibrosis and sclerosis by the administration of compositions containing A_1 adenosine receptor antagonists and/or P_{2X} purinoceptor antagonists, or combinations thereof.

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METHODS FOR THE PREVENTION AND TREATMENT OF FIBROSIS AND SCLEROSIS

This application claims the benefit of U.S. Provisional Application No. 60/072,896; filed 28 January 1998.

Field of the Invention

The present invention relates to methods of treating fibrosis and sclerosis, using A_1 adenosine receptor antagonists and P_{2X} purinoceptor antagonists, or combinations thereof.

Background

Purinergic receptors can be classified into the P_1 (adenosine) receptors and the P_2 (adenosine 5' triphosphate) receptors. Adenosine receptors can further be delineated into major subclasses, the A_1, A_2 (A_{2a} and A_{2b}) and A_3 adenosine receptors. These subtypes are differentiated by molecular structure, radioligand binding profiles, and by pharmacological activity and signal transduction mechanisms. Binding of adenosine, a naturally occurring nucleoside, to specific adenosine receptors leads to either stimulation (A_2-receptor activation) or inhibition (A_1-receptor activation) of adenylate cyclase activity resulting in an increase or decrease of intracellular cAMP, respectively. Most tissues and cell types possess either the A_1 or A_2 receptor, or both. Moreover, A_1 adenosine receptors have been identified in the nuclear fraction of splenocytes (Donnabella, *Life Sci.* 46:1293 (1990)). Specific A_1, A_2, and A_3 adenosine receptor antagonists and agonists are known. *See, e.g.*, Trivedi et al., *Structure-Activity Relationships of Adenosine A_1*

and A_2 Receptors, *In*: Adenosine and Adenosine Receptors, M.Williams, Ed., Humana Press, Clifton, New Jersey, USA (1990); Jacobson et al., *J. Medicinal Chem.* 35:407 (1992); Fredholm et al., *Pharm. Rev.* 46:143 (1994); Jacobson, Abstracts from Purines '96, *Drug Dev. Res.*, March 1996, page 112. Divalent ions (Mg^{2+} and Ca^{2+}) and allosteric enhancers enhance the binding of A_1 adenosine receptor agonists to A_1 adenosine receptors (Kollias-Baker, *Circ. Res.* 75:961 (1994)). Allosteric enhancers enhance A_1 receptor mediated responses and are described in Bhattacharya, *Biochim. Biophys. Acta* 1265:15 (1995).

Based on potency profiles of structural analogues for ATP, ATP-sensitive (P2) purinoceptors have been subclassified into P_{2X} and P_{2Y} purinoceptors. With few exceptions, P_{2X} receptors are located on vascular smooth muscle cells and mediate vasoconstriction and P_{2Y} receptors are located on endothelial cells and mediate vasodilation. Burnstock and Kennedy, *Gen. Pharmacol.* 16:433 (1985; Ralevic eta l., *Br. J. Pharmacol.* 103:1108 (1991). P_{2X} receptors are present on arteries of a number of different species. Bo and Burnstock, *J Vas Res* 30:87 (1993). The presence of P_{2X} purinoceptors on pulmonary arteries is reported in Neely, C.F., Am J Physiol 270:L889-L897, 1996.

Inflammatory cells, including monocytes and alveolar macrophages express the A_1, A_2 and A_3 adenosine receptor subtypes. Eppell et al., *J. Immunology* 143:4141 (1989); Lapin and Whaley, *Clin. Exp. Immunol.* 57:454 (1984); Saijadi, et äl., *J. Immunol.* 156:3435 (1996). The presence of A_1 adenosine receptors on human monocytes/macrophages is reported, e.g., in Salmon, J.E., J Immunology 151:2775-2785, 1993. Mature monocytes enter the circulatory system from the bone marrow; some monocytes enter tissues and develop into macrophages in the spleen, lymph nodes, liver, lung, thymus, peritoneum, nervous system, skin and other tissues. Monocytes and macrophages can be identified by morphology, cell surface antigens, and the presence of characteristic enzymes. Both monocytes and macrophages play a role in inflammatory responses and secrete various proteins active in immune and inflammatory responses, including Tumor Necrosis Factor (TNF) and Interleukin I (IL-1)). Upon stimulation, monocytes and

macrophages can generate various oxygen metabolites, including superoxide anion and H_2O_2 that are toxic to both pathogens and normal cells.

Fibroblasts are the major cell type responsible for the synthesis of collagen, a fibrous protein essential for maintaining the integrity of the extracellular matrix found in the dermis of the skin and other connective tissues. The production of collagen is a finely regulated process, and its disturbance may lead to the development of tissue fibrosis. The formation of fibrous tissue is part of the normal healing process after injury, including injury due to surgery. However, in some circumstances there is an abnormal accumulation of fibrous material such that it interferes with the normal function of the affected tissue.

Scar tissue serves only a structural role, but does not contribute to the function of the organ in which it appears. For example, as fibrotic scar tissue replaces heart muscle damaged by hypertension, the heart becomes less elastic and thus less able to do its job. Similarly, pulmonary fibrosis causes the lungs to stiffen and impairs lung function. Fibrotic growth can proliferate and invade healthy surrounding tissue, even after the original injury heals. In most cases fibrosis is a reactive process, and several different factors can apparently modulate the pathways leading to tissue fibrosis. Such factors include the early inflammatory responses, local increase in fibroblast cell populations, modulation of the synthetic function of fibroblasts, and altered regulation of the biosynthesis and degradation of collagen.

Stimulation of fibroblast activity is involved in the development of fibrotic conditions, including spontaneous and induced conditions. Abnormal accumulation of collagen in the extracellular matrix, resulting from excessive fibroblast proliferation and/or collagen production, can cause fibrosis of a number of tissues including the skin. Many common debilitating diseases, such as liver cirrhosis and pulmonary fibrosis, involve the proliferation of fibrous tissue as do certain skin diseases such as scleroderma, and the formation of adhesions, keloids, and hypertrophic scars.

Summary of the Present Invention

We have identified that fibrosis and/or sclerosis is associated with activation of A_1 adenosine receptors, and that administration of an A_1 adenosine receptor antagonist offers potential as an antifibrotic or antisclerotic treatment. The present invention accordingly provides a method of inhibiting fibrosis and/or sclerosis in a subject afflicted with a fibrosing or sclerosing disorder. The method comprises administering to the subject an effective fibrosis-inhibiting or sclerosis-inhibiting amount of an A_1 adenosine receptor antagonist.

We have identified that fibrosis and/or sclerosis is associated with activation of P_{2X} purinoceptors, and that administration of a P_{2X} purinoceptor antagonist offers potential as an antifibrotic or antisclerotic treatment. The present invention accordingly provides a method of inhibiting fibrosis and/or sclerosis in a subject afflicted with a fibrosing or sclerosing disorder. The method comprises administering to the subject an effective fibrosis-inhibiting or sclerosis-inhibiting amount of a P_{2X} purinoceptor antagonist.

The present invention further provides a method of inhibiting fibrosis and/or sclerosis in a subject afflicted with a fibrosing or sclerosing disorder, by administering an effective fibrosis-inhibiting or sclerosis-inhibiting amount of a composition containing at least one A_1 adenosine receptor antagonist and at least one P_{2X} purinoceptor antagonist.

A first aspect of the present invention is a method of treating fibrosis or sclerosis in a subject in need of such treatment, by administering a composition containing an A_1 adenosine receptor antagonist, a P_{2X} purinoceptor antagonist, or a combination of at least one A_1 adenosine receptor antagonist and at least one P_{2X} purinoceptor antagonist. The composition is administered in an amount that is effective in reducing the rate of fibrosis or sclerosis that would occur without treatment.

A further aspect of the present invention is a method of preventing fibrosis or sclerosis in a subject in need of such treatment, by administering a composition

containing an A_1 adenosine receptor antagonist, a P_{2X} purinoceptor antagonist, or a combination of at least one A_1 adenosine receptor antagonist and at least one P_{2X} purinoceptor antagonist. The composition is administered in an amount that is effective in reducing the formation of fibrotic or sclerotic tissue that would otherwise occur without treatment.

The foregoing and other objects and aspects of the present invention are explained in detail in the specification set forth below.

Detailed Description

It has now been found that administration of compositions containing A_1 adenosine receptor antagonists and/or P_{2X} purinoceptor antagonists, or a combination thereof, can prevent or inhibit fibrosis and/or sclerosis.

Endothelial injury is often due to non-infectious injurious agents, such as trauma or burns, exposure to drugs or chemical agents, immune-complex events, and ischemia-initiated events. Such injury, and the associated acute inflammatory response, is characterized by infiltration of neutrophils, adhesion of neutrophils to endothelial cells, hemorrhage, and the presence of macrophages. However, the exact mechanism by which these injurious agents induce endothelial injury is unknown. The neutrophil-derived release of reactive oxygen species may induce lipid peroxidation of biological membranes, and has been associated with an increase in collagen synthesis. Casini, *Hepatology* 25:361 (1997). The release of reactive oxygen species, and byproducts of lipid peroxidation may also promote the activation, migration, and adhesion of neutrophils to endothelial cells, and may prime macrophages for the release of cytokines and growth factors (cytokines include interleukin-1 (Il-1), interleukin-2 (Il-2), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α); growth factors include transforming growth factor-beta (TGF-β), insulin-like growth factor-I (IGF-I), and platelet-derived growth factors (PDGFs)). It has been reported that these growth factors are elevated in disease states associated with fibrosis and/or sclerosis, and may be, in part, responsible for the fibrosis and/or sclerosis.

It is known that lipopolysaccharide (LPS, endotoxin) binds to cells and induces the release of mediators from neutrophils, monocytes, macrophages, and endothelial cells. These mediators are important in the pathophysiology of endotoxin-induced acute lung injury. The present inventor has found that, in pulmonary arterial endothelial cells, both A_1 adenosine receptor agonists and endotoxin (LPS) induce thromboxane release, that endotoxin induced inhibition of adenylate cyclase or thromboxane release is blocked by a highly selective A_1 adenosine receptor antagonist (1,3 dipropyl-8-cyclopentylxanthine (DPCPX)), and that endotoxin displaces the binding of highly selective A_1 adenosine receptor antagonist radioligands [^{3}H] DPCPX and ^{125}I-BW A844U. These findings indicate that LPS binds to and activates A_1 adenosine receptors on pulmonary artery endothelial cells. Also, A_1 adenosine receptor antagonists are able to block such endotoxin-induced lung injury, supporting that activation of A_1 adenosine receptors is important in endotoxin-induced acute lung injury. Neely, *Am J. Physiol., Lung Cell Mol. Physiol.*, 268:L1036, 1995. In the lung, LPS produced injury is characterized by the presence of neutrophils, macrophages and red blood cells in alveoli, and by alveolar edema and necrosis. Selective A_1 adenosine receptor antagonists are effective in preventing LPS-induced lung injury (Neely, *Am J Physiol* 272:L353 (1997).

The present inventor has determined that A_1 adenosine receptors and P_{2X} purinoceptors are also implicated in the pathogenesis of fibrosis and sclerosis. The present methods utilize A_1 adenosine receptor antagonists or P_{2X} purinoceptor antagonists, or combinations thereof, to block the cellular release of factors that act in the pathogenesis of fibrosis and/or sclerosis.

The present invention provides methods of preventing and treating fibrosis and sclerosis, wherein an effective amount of an A_1 adenosine receptor antagonist, a P_{2X} purinoceptor antagonist, or a combination thereof, is administered to a subject in need of such treatment. A single compound that antagonizes both the A_1 receptor and the P_{2X} purinoceptor may also be used in the methods of the present invention. An effective amount is that amount able to reduce the amount of fibrotic or sclerotic

growth that would occur in the absence of said antagonists, or slow the progress (over time) of fibrosis or sclerosis compared to that which would occur in the absence of said antagonists. In other words, the term "effective amount" refers to a concentration of an A_1 adenosine receptor antagonist, P_{2X} purinoceptor antagonist, or combination thereof, which is sufficient to interfere with the progression of fibrotic or sclerotic pathological changes. Preferably, the A_1 adenosine receptor antagonist is a selective A_1 adenosine receptor antagonist. Preferably, the P_{2X} purinoceptor antagonist is a selective P_{2X} purinoceptor antagonist.

Disease states characterized by acute and chronic inflammation and progressive fibrosis or sclerosis include autoimmune diseases (rheumatoid arthritis, Crohn's disease, scleroderma, glomerulonephritis, and progressive systemic sclerosis); irradiation induced fibrosis; fibrosis of the heart following myocardial infarction or ischemia-reperfusion injury; fibrosis of the lung associated with adult respiratory distress syndrome, irradiation injury, immune-complex disease, inhalation of chronic irritants, or chemotherapeutic agents; cirrhosis of the liver; chronic graft rejection of transplanted organs; fibrosis of the skin following thermal exposure; gingival periodontal fibrosis; cataract formation; keloid and adhesion formation following surgery and trauma; and arteriosclerosis/atherosclerosis. Okada, *Acta Pathologica Japonica* 43:160 (1993); Nishi *Br J Ophthalmology* 80:63 (1996); Kuroki *Br J Rheumatology* 34:31 (1995); Nakao *J Dental Res* 74:1072 (1995); Herman *Clinical Nephrology* 46:34 (1996); Thornton *Clin Exp Immunology* 103:67 (1996); Garner *J Investigative Dermatology* 101:875 (1993); Salmon-Her *Archives of Dermatology* 132:802 (1996); Liao *J Lab Clin Med* 128:452 (1996).

Fibrosis is the formation of fibrous tissue, usually as a reparative or a reactive process. As used herein, "fibrosis" does not refer to the formation of fibrous tissue that is a normal part of an organ or tissue, but includes those disorders or disease states that are caused by or accompanied by the abnormal deposition of scar tissue. Fibrosis can follow surgery in the form of adhesions, keloid tumors or hypertrophic (very severe) scarring. Fibrosis causes contractures and joint dislocation following severe burns, wounds or orthopedic injuries; it can

occur in any organ and accompanies many disease states, such as hepatitis (liver cirrhosis), hypertension (heart failure), tuberculosis (pulmonary fibrosis), scleroderma (fibrotic skin and internal organs), diabetes (nephropathy) and atherosclerosis (fibrotic blood vessels). Fibrosis also includes all arteriosclerotic disorders, pulmonary fibrosis, adult respiratory distress syndrome, inflammatory disorders including autoimmune disorders, sclerodermas, cirrhosis, keloids, adhesions and hypertrophic scars.

Keloids are firm, non-encapsulated, usually linear mass of hyperplastic scar tissue. Keloids comprise relatively large and fairly parallel bands of densely collagenous material, separated by bands or cellular fibrous tissue. Keloids commonly occur in the dermis and adjacent subcutaneous tissue, often after a traumatic injury, surgery or a burn. Keloids may be surgically removed, but often recur at the same site after surgical removal.

Sclerosis refers to an induration or hardening of chronic inflammatory origin. Hyperplasia of the interstitial fibrous or glial connective tissue can lead to induration of nervous system structures.

Skeletal muscle fibrosis is a phenomenon which frequently occurs in diseased or damaged muscle. It is characterized by the excessive growth of fibrous tissue, and impairs muscle function. The amount of muscle function loss generally increases with the extent of fibrosis. Disorders which typically result in skeletal muscle fibrosis include, for example, muscular dystrophies, such as Duchenne's muscular dystrophy and Becker's muscular dystrophy; and neuromuscular diseases, such as acute polyneuritis, poliomyelitis, Werdig/Hoffman disease, amyotrophic lateral sclerosis, and progressive bulbar atrophy. Such conditions also include traumatic denervation atrophy induced by either trauma or by neuromuscular disorders. Skeletal muscle fibrosis is often progressive. The present invention provides a method of treating skeletal muscle fibrosis in subjects, preferably mammals, in need of such treatment. The method is effective for reducing the extent of, or preventing the progression of, skeletal muscle fibrosis in a subject suffering from a disorder which targets skeletal muscle tissue. The treatment

includes administering to the individual a pharmaceutical composition containing an A_1 adenosine receptor antagonist, a P_{2X} purinoceptor antagonist, or a combination thereof, in an amount effective to reduce the rate of skeletal muscle fibrotic tissue growth.

Cardiovascular disease states involving fibrosis that can be treated by the methods of the present invention include left ventricular hypertrophy secondary to hypertension; and fibrosis associated with myocardial infarction, myocarditis, or with ischemia-reperfusion injury to the heart. The present invention provides a method of treating cardiac muscle fibrosis or cardiovascular fibrosis (e.g., arteriosclerotic changes in coronary arteries) in subjects in need of such treatment. The treatment includes administering to the individual a pharmaceutical composition containing an A_1 adenosine receptor antagonist, a P_{2X} purinoceptor antagonist, or a combination thereof, in an amount effective to reduce the rate of cardiac muscle fibrotic tissue growth, or arteriosclerotic or fibrotic changes in the vasculature.

Dermal fibrosing disorders include, but are not limited to, scleroderma, morphea, keloids, hypertrophic scars, familial cutaneous collagenoma, and connective tissue nevi of the collagen type. The present invention provides a method of treating dermal fibrosis in subjects in need of such treatment. The method is effective for reducing the extent of, or preventing the progression of, dermal fibrosis. The treatment includes administering to the individual a pharmaceutical composition containing an A_1 adenosine receptor antagonist, a P_{2X} purinoceptor antagonist, or a combination thereof, in an amount effective to reduce the rate of dermal fibrotic tissue growth. Administration may include topical or transdermal administration or injection into the affected area.

Fibrosis of internal organs (e.g., liver, lung, kidney, heart, blood vessels, gastrointestinal tract), occurs in disorders such as pulmonary fibrosis, liver cirrhosis, and scar formation. The present invention provides a method of treating fibrosis in internal organs in subjects in need of such treatment. The method is effective for reducing the extent of, or preventing the progression of, fibrotic changes in the target organ. The treatment includes administering to the individual a

pharmaceutical composition containing an A_1 adenosine receptor antagonist, a P_{2X} purinoceptor antagonist, or a combination thereof, in an amount effective to reduce the rate of internal organ fibrotic tissue growth. Administration may vary depending on the target organ, e.g., aerosol inhalation may be used to treat pulmonary fibrosis, whereas parenteral administration may be used to treat fibrosis of the liver.

Fibrotic conditions of the eye include conditions such as diabetic retinopathy, postsurgical scarring (for example, after glaucoma filtering surgery), and proliferative vitreoretinopathy. The present invention provides a method of treating opthalmic fibrosis in subjects in need of such treatment. The method is effective for reducing the extent of, or preventing the progression of, fibrotic changes in the eye. The treatment includes administering to the individual a pharmaceutical composition containing an A_1 adenosine receptor antagonist, a P_{2X} purinoceptor antagonist, or a combination thereof, in an amount effective to reduce the rate of ophthalmic fibrotic tissue growth. Treatment may include intra-ocular administration or topical administration of the active compound.

In rheumatoid arthritis, a characteristic feature is persistent inflammatory synovitis of the peripheral joints, leading to cartilage destruction and bone erosions. Rheumatoid synovitis is characterized by an increased number of synovial lining cells and perivascular infiltration with mononuclear cells. Activated synovial fibroblasts are common, particularly at the interface of bone and cartilage. The rheumatoid synovium is characterized by the presence of cytokines, chemokines and other products secreted by activated lymphocytes, macrophages, and fibroblasts. Bone and cartilage destruction occurs in juxtaposition to the inflamed synovium, or pannus, that spreads to cover the articular cartilage. The present invention provides a method of treating rheumatoid arthritis in subjects in need of such treatment. The method is effective for reducing the extent of, or preventing the progression of, articular damage. The treatment includes administering to the individual a pharmaceutical composition containing an A_1 adenosine receptor antagonist, a P_{2X} purinoceptor antagonist, or a combination thereof, in an amount

effective to ameliorate symptoms or reduce the rate of articular damage. Treatment may include intra-articular administration of the active compound to a specific affected joint.

The phrase "fibrotic disorder" or "sclerotic disorder" means diseases, conditions or other abnormal medical states which typically result in fibrosis or sclerosis, respectively. The phrase "suffering from" such a disorder means that the subject exhibits symptoms of an aforementioned disorder and thus is likely to develop significant pathological fibrosis or sclerosis in the course of events, even though signs of fibrosis or sclerosis may not be evident at the time of diagnosis. The diagnosis of individuals who suffer from disorders that typically result in debilitating fibrosis or sclerosis may be readily made by those having ordinary skill in the art using well established criteria and methods.

The range of dosages and the frequency of delivery of a composition according to the present invention, to be effective in treating or preventing fibrosis or sclerosis, can be determined by those having ordinary skill in the art. A methodology for determining appropriate dosage in treating skeletal muscle fibrosis, for example, includes determining the existing state of skeletal muscle fibrosis of a patient; administering at a preselected frequency, a preselected amount of pharmaceutical formulation containing A_1 adenosine receptor antagonists and/or P_{2X} purinoceptor antagonists; determining the state of skeletal muscle fibrosis exhibited by the patient at a later time when the disorder, if untreated, would have increased fibrotic tissue development; and adjusting the dosage amount and/or delivery rate to reduce, maintain or increase the effect of preventing or reducing fibrosis. A number of methods are available to determine the state of skeletal muscle fibrosis of a patient, including evaluating a muscle tissue biopsy from the subject by histochemical or immuno-histochemical stains that can detect fibrotic tissue. Examples of histochemical stains include, for example, hematoxylin and eosin (H & E), trichrome and ATPase (at pH 4.3, 4.65 and 10.4). Representative antibodies that can be used to label muscle fibers for immuno-histochemical staining include, for example, myosin, type IV collagen, laminin, and fibronectin.

Subjects to be treated by the method of the present invention include, but are not limited to, subjects afflicted with a dermal fibrosing disorder, a skeletal muscle fibrosing disorder, fibrosis of an internal organ, cardiovascular fibrosis, fibrosis due to autoimmune disease, and fibrotic conditions of the eye.

Subjects to be treated by the method of the present invention include both human and animal (e.g., dog, cat, cow, horse) subjects, and are preferably mammals. Subjects include those diagnosed with a fibrosing or sclerosing disorder, in which it is desired to inhibit, prevent or reduce fibrotic or sclerotic changes. Subjects also include those scheduled to undergo surgery, where the methods may be employed prophylactically (prior to surgery or following surgery, but prior to the occurrence of fibrosis or sclerosis), e.g., to prevent or reduce keloid, adhesion, or hypertrophic scar formation.

Effective dosages of the active compounds in the present methods will vary depending on the subject to be treated (body weight, age, general health), route and timing of administration, type of formulation, characteristics of the compounds used, the severity of disease, concurrent therapies, and the desired effect. These amounts can be determined by pharmacokinetic principles well known to those skilled in the art.

Agents which bind to A_1 adenosine receptors are well known to those of skill in the art. One of the best known classes of adenosine receptor antagonists are the xanthines, which include caffeine and theophylline. See e.g., Müller et al., *J. Med. Chem.* 33:2822 (1990). Both agonists and antagonists have been synthesized for A_1 adenosine receptors. For example, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) is a highly selective A_1 adenosine receptor antagonist with negligible nonspecific binding (less than 1%) in tissues (Jacobson et al., *J. Med. Chem.* 35:407 (1992); Bruns, RF "Adenosine Receptor Binding Assays", Receptor Biochemistry and Methodology, Volume II: Adenosine Receptors, DMF Cooper and C. Londos (Eds.), Alan Liss, Inc., New York, NY 1988, pp. 43-62). Other examples of A_1 adenosine receptor antagonists include, but are not limited to, xanthine amine congener (XAC); xanthine carboxylic congener (XCC); 1,3-

dipropyl-xanthines such as 1,3-dipropyl-8-(-3-noradamantyl) xanthine (KW 3902), 1,3-dipropyl-8-(dicyclopropylmethyl) xanthine (KF 15372), 1,3-dipropyl-8-[2-(5,6-epoxy)norbonyl]xanthine (ENX), 8-(1-aminocyclopentyl)-1,3-dipropylxanthine (IRFI 117), 1,3-dipropyl-8-(3-noradamantyl) xanthine (NAX) and 1,3-dipropyl-8-(3-oxocyclopentyl) xanthine (KFM 19); 1-propyl-3-(4-amino)-3-phenethyl)-8-cyclopentylxanthine (BW-A844U); 1,3-dipropyl-8-sulfophenylxanthine (DPSPX); cyclopentyl theophylline (CPT) and 7-[2-ethyl (2-hydroxyethyl)amino]-ethyl]-3,7-dihydro-1,3-dimethyl-8-(phenylmethyl)-1H-purine-2, 6-dione (Bamifylline); N^6 , 9-methyl adenines such as (±)-N^6-endonorbornan-2-yl-9-methyladenine (N-0861) and 8-(N-methylisopropyl) amino- N^6- (5'-endohydroxy-endonorbornyl)-9-methyladenine (WRC-0571); N^6, 9-disubstituted adenines; 2-phenyl-7-deazaadenines such as (R)-7,8-dimethyl-2-phenyl-9-(1-phenylethyl)-7-deazaadenine; 7,8-dihydro-8-ethyl-2-(3-noradamantyl)-4-propyl-1H-imidazo[2,1-*i*]purin-5(4*H*)-one; (±)R-1-[(,)-3[2-[phenylpyrazolo (1,5-a) pyridin-3-yl]acryloyl]-2-piperidine ethanol; 8-azaxanthines such as 7-cyclopentyl-1,3-dipropyl-8-azaxanthine; tetrahydrobenzothiophenones such as ethyl-3-(benzylthio)-4-oxo-4,5,6,7-tetrahydrobenzo[c]thiophene-1-carboxylate; N-6-cyclopentyl-3'-substituted xylo-furanosyl adenosines (Van Calinbergh, *J. Med. Chem.* 40:3765, November 1997).

Additionally, selective analogues of adenosine receptor antagonists have been developed through the "functionalized congener" approach. Analogues of adenosine receptor ligands bearing functionalized chains have been synthesized and attached covalently to various organic moieties such as amines and peptides. Jacobson et al. *J. Med. Chem.* 35:408 (1992) has proposed various derivatives of adenosine and theophylline for use as receptor antagonists.

Antibodies raised against the A_1 adenosine receptor that selectively target and bind to this receptor can also be used as A_1 adenosine receptor antagonists. Such antibodies targeted to the A_1 adenosine receptor can be produced routinely in accordance with well known methods of antibody production. As used herein, the term "A_1 adenosine receptor antagonist" encompasses antibodies that selectively or

specifically bind to the receptor, when such antibodies are used for their antagonist effects.

P_{2X} purinoceptor antagonists are known in the art; an example of a selective P_{2X} purinoceptor antagonist is pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS). Additional specific pharmacological antagonists of purinoceptors have been described by Humphrey et al., *Naunyn-Schmied. Arch. Pharmacol.* 352:585 (1995); Abracchio and Burnstock, *Pharmac. Ther.* 64:445 (1994); Bultmann et al., *Naunyn-Schmied. Arch. Pharmacol.* 354:481 (1996); and Bultmann et al., *Naunyn-Schmied. Arch. Pharmacol.* 354:498 (1996). Antibodies raised against the P_{2X} purinoceptor that selectively target and bind to this receptor can also be used as P_{2X} purinoceptor antagonists. Such antibodies targeted to the P_{2X} purinoceptor can be produced routinely in accordance with well known methods of antibody production. As used herein, the term " P_{2X} purinoceptor antagonist" encompasses antibodies that selectively or specifically bind to the receptor, when such antibodies are used for their antagonist effects.

As used herein, an amount of a compound that is effective for treating fibrosis and/or sclerosis is that which: inhibits the further progression of fibrotic or sclerotic changes that would otherwise occur; that which reduces the rate of fibrosis or sclerosis; or that which prevents fibrosis or sclerosis from occurring. Compositions of A_1 receptor antagonists and P_{2X} purinoceptor antagonists used in the present methods may further comprise a pharmaceutically acceptable carrier, including but not limited to saline, water, dextrose and water, cyclodextrins or similar sugar solutions, low dose sodium hydroxide solutions, propylene glycol, and polyethylene glycol.

The pharmaceutical composition may be employed, as an example, in oral dosage form as a liquid composition. Such liquid compositions can include suspension compositions or syrup compositions and can be prepared with such carriers as water; a saccharide such as sucrose, sorbitol, fructose, and the like; a glycol such as polyethyleneglycol, polypropyleneglycol, and the like; an oil such as sesame oil, olive oil, soybean oil, and the like; an antiseptic such as p-hydroxy-

benzoic acid esters and the like; and a flavor component such as a fruit flavor or a mint flavor. The pharmaceutical composition may also be in the form of powder, pills, capsules, and tablets and can be prepared with various carriers. Suitable carriers include, but are not limited to, lactose, glucose, sucrose, mannitol, and the like; disintegrators such as starch, sodium alginate, and the like; binders such as polyvinyl alcohol, hydroxypropyl cellulose, gelatin, and the like; surfactants such as, for example, fatty acid esters; and plasticizers such as, for example, glycerins. It should be noted that in the preparation of the tablets and capsules, a solid pharmaceutical carrier is used. The pharmaceutical composition may be used in the form of an aerosol where advantageous, such as in the treatment of pulmonary fibrosis.

The pharmaceutical compositions also may be formulated as an injectable solution. These compositions are prepared using appropriate sterile aqueous solutions which may include, but are not limited to, water, saline, dextrose and water or other similar sugar solutions, and buffer additives, as will be apparent to one skilled in the art.

The formulations of the present invention further include those suitable for topical or transdermal administration, ophthalmic, parenteral (including subcutaneous, intramuscular and intravenous), oral, inhalational, or nasal administration. Topical formulations comprise the active compound dissolved or suspended in one or more media such as mineral oil, petroleum, polyhydroxy alcohols or other bases used for topical pharmaceutical formulations. The addition of other accessory ingredients may also be desirable. Ophthalmic formulations comprise purified aqueous solutions of the active compounds with preservative agents and isotonic agents. The formulations are preferably adjusted so that the pH and isotonic factors match that of the eye.

It is to be understood that the choice of formulation may vary depending on the specific compound utilized. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy.

Pharmaceutical compositions that include A_1 adenosine receptor antagonists and/or P_{2X} purinoceptor antagonists may be administered by any method that can deliver the composition to the site in the body of a subject where anti-fibrotic or anti-sclerotic treatment is desired. These methods include but are not limited to oral, subcutaneous, transdermal, inhalational or aerosol, intravenous, intramuscular, intra-articular, intra-thecal, liposomal and parenteral methods of administration. In order to treat fibrosis confined to a specific site, a site-specific method of delivery is preferred, such as infusion directly to an organ. For example, to treat fibrosis associated with denervation atrophy caused by traumatic injury to one or a group of nerves affecting muscles in a localized region of the body, delivery of the pharmaceutical composition directly to the affected muscles, such as by intramuscular injection, may be used to advantage. Methods of administering compositions in a site-specific manner will vary depending on the affected site. Appropriate methods of site-specific delivery will be readily apparent to one skilled in the art. When fibrosis is associated with disease that affects tissues throughout the body, a systemic delivery method, such as intravenous infusion or subcutaneous delivery is desirable.

The following examples are provided to illustrate the present invention, and should not be construed as limiting thereof.

EXAMPLE 1

Effect of A_1 Adenosine Receptor Antagonist on Experimentally Induced Liver Fibrosis in Rats

Hepatic fibrosis is induced in a group of male Spague Dawley rats by intraperitoneal injection of dimethylnitrosamine, as is known in the art (e.g., 1% DMN dissolved in saline solution at 10 mg/kg/day for 3 consecutive days per week for 2 weeks, for rats of 150-200 g body weight).

Animals are divided into three groups: Group A animals receive DMN (10 mg/kg/day) for 3 consecutive days a week for 2 weeks. This dose and treatment

schedule with DMN is reported to produce hepatic fibrosis; Mancini, *Virchows Archiv* 424:25-31, 1994.

Group B animals receive the A_1 adenosine receptor antagonist (±) N6-(*endo*-2-Norbornyl)-9-methyladenine (N0861) as an intravenous bolus (2 mg/kg) prior to DMN exposure, plus as a continuous infusion of N0861 (0.02 mg/kg/min) at the time DMN treatment is begun and continued for 2 weeks. The continuous intravenous infusion may be achieved, e.g., via a mini osmotic pump inserted under the skin of the back. *See* Peck and Cusack, *Drugs of the Future* 18:433-435, 1993.

Group C animals receive only N0861 as a bolus (2mg/kg) plus a continuous infusion of N0861 (0.02 mg/kg/min) for 2 weeks and are a control group.

After 2 weeks, animals are sacrificed and the livers removed. Hepatic fibrosis is quantitated using methods as known in the art, e.g., 1) a quantitative colorimetric assay for collagen; 2) immunohistochemistry-immunoperoxidase staining for alpha-SM actin and for laminin; and/or 3) histomorphometric analysis of inflammatory infiltrate and fibrosis. Mancini, *Virchows Archiv* 424:25-31, 1994..

Following sacrifice, resident peritoneal macrophages in each rat are isolated and cultured protein levels of PDGF-AA, PDGF-BB, and TGF-beta 1 are determined using techniques available in the art, such as by immunoblot analysis. Kovacs, *Immunobiology* 190:263-274, 1994.

EXAMPLE 2

Effect of P_{2X} Purinoceptor Antagonist on Experimentally Induced Liver Fibrosis in Rats

Hepatic fibrosis is induced in a group of male Spague Dawley rats as described in example 1 above. Animals are divided into three treatment groups.

The P_{2X} purinoceptor antagonist pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) is administered as an intravenous bolus (15 mg/kg) every 4 hours.

Group A animals receive DMN (10 mg/kg/day) for 3 consecutive days a week for 2 weeks. This dose and treatment schedule with DMN is known to produce hepatic fibrosis. Mancini, *Virchows Archiv* 424:25-31, 1994.

Group B animals receive PPADS as an intravenous bolus (15 mg/kg) every 4 hours at the time DMN treatment is begun, and for 2 weeks thereafter.

Group C animals receive only PPADS as an intravenous bolus (15 mg/kg) every 4 hours for 2 weeks and represent the control group.

After 2 weeks, animals are sacrificed and their livers removed. Hepatic fibrosis is quantitated using methods known in the art, such as 1) a quantitative colorimetric assay for collagen; 2) immunohistochemistry-immunoperoxidase staining for alpha-SM actin and for laminin; and/or 3) histomorphometric analysis of inflammatory infiltrate and fibrosis. Mancini Virchows Archiv 424:25-31, 1994.

Also following sacrifice, resident peritoneal macrophages are isolated from each animal and cultured protein levels of PDGF-AA, PDGF-BB, and TGF-beta 1 are determined using techniques available in the art, such as by immunoblot analysis. Kovacs, *Immunobiology* 190:263-274, 1994.

EXAMPLE 3

Effect of Combined A_1 Adenosine Receptor Antagonist and P_{2X} Purinoceptor Antagonist on Experimentally Induced Liver Fibrosis in Rats

Hepatic fibrosis is induced in male Spague Dawley rats as described above.

Each animal receives the A_1 adenosine receptor antagonist, (±) N6-(*endo*-2-Norbornyl)-9-methyladenine (N0861) administered as an intravenous bolus (2 mg/kg) and as a continuous intravenous infusion (0.02 mg/kg/min); each animal additionally receives the P_{2X} purinoceptor antagonist pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) as an intravenous bolus (15 mg/kg) every 4 hours, at the time DMN treatment is begun. Animals are divided into three treatment groups.

Group A animals receive DMN (10 mg/kg/day) for 3 consecutive days a week for 2 weeks. This dose and treatment schedule with DMN is reported to produce hepatic fibrosis. Mancini, *Virchows Archiv* 424:25-31, 1994.

Group B animals receive N0861 as an intravenous bolus (2 mg/kg) plus as a continuous intravenous infusion (0.02 mg/kg/min), and receive PPADS (15 mg/kg) every 4 hours at the time DMN treatment is begun and continued for 2 weeks.

Group C animals receive only N0861 as a bolus (2 mg/kg) plus a continuous intravenous infusion (0.02 mg/kg/min) plus PPADS (15 mg/kg) every 4 hours for 2 weeks and represent the control group.

After 2 weeks, animals are sacrificed and their livers removed. Hepatic fibrosis will be quantitated with the use of 1) a quantitative colorimetric assay for collagen; 2) immunohistochemistry-immunoperoxidase staining for alpha-SM actin and for laminin; and 3) histomorphometric analysis of inflammatory infiltrate and fibrosis. Mancini, R., Virchows Archiv 424:25-31, 1994.

Also, following sacrifice resident peritoneal macrophages will be isolated and cultured protein levels for PDGF-AA, PDGF-BB, and TGF-beta 1 will be determined with the use of immunoblot analysis. Kovacs, E.J., Immunobiol 190:263-274, 1994.

EXAMPLE 4

Effect of A_1 Adenosine Receptor Antagonist on Experimentally Induced Arteriosclerotic Changes in Pigs

Domestic pigs are sedated, anesthetized, intubated and ventilated using techniques that are known in the art. Under aseptic conditions, a left thoracotomy is performed and the proximal segments of the left anterior descending (LAD) and circumflex coronary artery (LCX) are carefully dissected. The dissected segments are gently wrapped with cotton mesh soaked in 0.05 ml suspension of recombinant human IL-β (2.5 μg) bound to sepharose beads. This technique is reported to produce arteriosclerotic changes in coronary arteries in pigs; Ito, *J Clin Invest* 96:1288-1294, 1995.

Animals are divided into 3 treatment groups. In Group A animals, the coronary arteries are treated with cotton mesh soaked in 0.05 ml suspension of recombinant human IL-β (2.5 μg) bound to sepharose beads.

In Group B animals, prior to the thoracotomy, an A_1 adenosine receptor antagonist, (±) N6-(*endo*-2-Norbornyl)-9-methyladenine (N0861) is administered as an intravenous bolus (2 mg/kg) plus as a continuous intravenous infusion (0.02 mg/kg/min). After the continuous intravenous infusion is started, the thoracotomy as described above is performed, and the coronary arteries are dissected and treated with cotton mesh soaked in 0.05 ml suspension of recombinant human IL-β (2.5 μg) bound to sepharose beads. The continuous infusion of N0861 is maintained for two weeks.

In Group C, animals receive only N0861 bolus (2 mg/kg) plus a continuous infusion of N0861 (0.02 mg/kg/min) for 2 weeks.

After 2 weeks the animals are sacrificed and the hearts are removed. The coronary arteries are fixed by perfusion with saline and 6% formaldehyde. After fixation the arteries are cut transversely into segments at approximately 5-mm intervals and these segments are stained for photomicroscopy to quantitate the degree of intimal thickening. *See, e.g.,* Ito, *J Clin Invest* 96:1288-1294, 1995. In addition to quantitating intimal thickening of these segments, protein levels for PDGF-AA, PDGF-BB, and TGF-beta 1 are determined using immunoblot analysis as is known in the art (*see* Kovacs, *Immunobiol* 190:263-274, 1994).

EXAMPLE 5

Effect of P_{2X} Purinoceptor Antagonist on Experimentally Induced Arteriosclerotic Changes in Pigs

Arteriosclerotic changes in coronary arteries in pigs are produced as described above. The pigs are divided into three treatment groups.

In Group A animals, coronary arteries are treated with cotton mesh soaked in 0.05 ml suspension of recombinant human IL-β (2.5 μg) bound to sepharose beads.

In Group B animals, prior to the thoracotomy, the P_{2X} purinoceptor antagonist pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) is administered as an intravenous bolus (15 mg/kg) every 4 hours for 2 weeks. After administration of PPADS is begun, the thoracotomy is performed, and the coronary arteries are dissected and treated with cotton mesh soaked in 0.05 ml suspension of recombinant human IL-β (2.5 μg) bound to sepharose beads.

In Group C, animals receive only PPADS as an intravenous bolus (15 mg/kg) every 4 hours for 2 weeks and represent the control group.

After 2 weeks the animals are sacrificed and the hearts are removed. The coronary arteries are perfused with saline and 6% formaldehyde. After fixation the arteries are cut transversely into segments at 5-mm intervals and these segments are stained for photomicroscopy to quantitate the degree of intimal thickening. Ito, *J Clin Invest* 96:1288-1294, 1995. In addition to quantitating intimal thickening of these segments, protein levels for PDGF-AA, PDGF-BB, and TGF-beta 1 are determined using immunoblot analysis. *See, e.g.*, Kovacs, *Immunobiol* 190:263-274, 1994.

EXAMPLE 6

Effect of Combined A_1 Adenosine Receptor Antagonist and P_{2X} Purinoceptor Antagonist on Experimentally Induced Arteriosclerotic Changes in Pigs

Arteriosclerotic changes are induced in domestic pigs' arteries as described above. The pigs are divided into three treatment groups.

In Group A animals, coronary arteries are treated with cotton mesh soaked in 0.05 ml suspension of recombinant human IL-β (2.5 μg) bound to sepharose beads.

In Group B animals, prior to the thoracotomy, an A_1 adenosine receptor antagonist, (±) N6-(*endo*-2-Norbornyl)-9-methyladenine (N0861) is administered as an intravenous bolus (2 mg/kg) plus as a continuous intravenous infusion (0.02 mg/kg/min); the P_{2X} purinoceptor antagonist pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) is also administered as an intravenous bolus (15 mg/kg) every 4 hours for 2 weeks. Following the start of N0861 and PPADS administration, the thoracotomy is performed, and the coronary arteries are dissected and treated with cotton mesh soaked in 0.05 ml suspension of recombinant human IL-β (2.5 μg) bound to sepharose beads.

In Group C, animals receive N0861 as a bolus (2 mg/kg) plus as a continuous intravenous infusion (0.02 mg/kg/min); and also receive PPADS (15 mg/kg) every 4 hours for 2 weeks. These animals are the control group.

After 2 weeks the animals are sacrificed and the hearts are removed. The coronary arteries are perfused with saline and 6% formaldehyde. After fixation the arteries are cut transversely into segments at 5-mm intervals and these segments are stained for photomicroscopy to quantitate the degree of intimal thickening. Ito, *J Clin Invest* 96:1288-1294, 1995. In addition to quantitating intimal thickening of these segments, protein levels for PDGF-AA, PDGF-BB, and TGF-beta 1 is determined with the use of immunoblot analysis. Kovacs, *Immunobiol* 190:263-274, 1994.

EXAMPLE 7

Experimentally Induced Dermal Fibrosis in Mice

Human keloids are implanted in athymic mice to create an animal model of human keloids. *See* Waki et al., *Arch. Otolaryngol. Head Neck Surg.* 117:1177 (1991). Mice are divided into four treatment groups and keloids are measured. One group is administered an A_1 adenosine receptor antagonist, by injection into the keloid or by topical or transdermal administration. A second group is treated with an identical regime, but using a P_{2X} purinoceptor antagonist. A third group is treated with an identical regime, but using a combination of the A_1 adenosine

receptor antagonist and the P_{2X} purinoceptor antagonist. A fourth group is maintained as a control group, receiving either no treatment, or treatment with a placebo.

After treatment is concluded, keloids are assessed by measurement, biopsy and autopsy to determine the rate of growth or rate of regression of keloids.

THAT WHICH IS CLAIMED IS:

1. A method of treating a disorder that results in fibrosis or sclerosis, in a subject in need of such treatment, comprising administering to said subject a composition selected from the group consisting of:

(a) A_1 adenosine receptor antagonists;

(b) P_{2X} purinoceptor antagonists; and

(c) a combination of at least one A_1 adenosine receptor antagonist and at least one P_{2X} purinoceptor antagonist;

wherein said composition is administered in an amount effective to reduce the rate of fibrosis or sclerosis.

2. The method of claim 1 wherein said disorder is selected from skeletal muscle fibrosis, irradiation-induced fibrosis, autoimmune-related fibrosis, cardiovascular fibrosis, arteriosclerotic disorders, pulmonary fibrosis, adult respiratory distress syndrome, inflammatory disorders, scleroderma, cirrhosis, keloids, adhesions and hypertrophic scars.

3. A method of claim 1 wherein said disorder is skeletal muscle fibrosis associated with a condition selected from muscular dystrophy, denervation atrophy induced by neuromuscular disease, and traumatic injury-induced denervation atrophy.

4. A method according to claim 1 wherein said disorder is cardiovascular fibrosis selected from left ventricular hypertrophy secondary to hypertension, fibrosis associated with myocardial infarction, fibrosis associated with ischemia-reperfusion injury, and fibrosis associated with myocarditis.

5. A method according to claim 1, wherein said disorder is a dermal fibrosis.

6. A method according to claim 1,wherein said disorder is selected from keloid formation, hypertrophic scar formation, or adhesion formation.

7. A method according to claim 1, wherein said disorder is an ophthalmic fibrosis.

8. A method according to claim 1, wherein said composition is administered topically.

9. A method according to claim 1, wherein said composition is administered parenterally.

10. A method according to claim 1, wherein said disorder is a dermal fibrosis and said composition is administered topically.

11. A method according to claim 1, wherein said disorder is pulmonary fibrosis and said composition is administered by inhalation.

12. A method according to claim 1, wherein said disorder is rheumatoid arthritis and said composition is administered by intra-articular injection.

13. A method according to claim 1, wherein said composition is administered directly to the affected anatomic site.

14. A method according to claim 1, wherein said composition is administered prophylactically.

15. A method according to claim 1, wherein said A_1 adenosine receptor antagonist is an antibody that binds to the A_1 adenosine receptor.

16. A method according to claim 1, wherein said P_{2X} purinoceptor antagonist is an antibody that binds to the P_{2X} purinoceptor.

17. A method of preventing fibrosis or sclerosis in a subject in need of such treatment, comprising administering to said subject a composition selected from the group consisting of:

(a) A_1 adenosine receptor antagonists;

(b) P_{2X} purinoceptor antagonists; and

(c) a combination of at least one A_1 adenosine receptor antagonist and at least one P_{2X} purinoceptor antagonist;

wherein said composition is administered in an amount effective to reduce the formation of fibrotic or sclerotic tissue that would occur in the absence of such treatment.

18. A method according to claim 17, wherein said fibrosis or sclerosis is due to keloid formation, hypertrophic scar formation, or adhesion formation.

19. A method according to claim 17, wherein said fibrosis is pulmonary fibrosis.

20. A method according to claim 19, wherein said pulmonary fibrosis is due to adult respiratory distress syndrome or irradiation induced fibrosis.

21. A method according to claim 17, wherein said composition is administered prior to scheduled surgery.

22. A method according to claim 17, wherein said A_1 adenosine receptor antagonist is an antibody that binds to the A_1 adenosine receptor.

23. A method according to claim 17, wherein said P_{2X} purinoceptor antagonist is an antibody that binds to the P_{2X} purinoceptor.

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(57) **Abstract**

Methods of treating or preventing fibrosis and sclerosis by the administration of compositions containing A_1 adenosine receptor antagonists and/or P_{2X} purinoceptor antagonists, or combinations thereof.

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INTERNATIONAL SEARCH REPORT

Int. ional Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/395 A61K31/52 A61K31/44 //(A61K31/52,31:44)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 26728 A (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) 12 October 1995 (1995-10-12) page 12, line 13 - line 18; claims 1,8,14-18 ---	1,2,4,8, 9,13,14, 17,19,21
X	EIDELMAN O ET AL: "A1 adenosine - receptor antagonists activate chloride efflux from cystic fibrosis cells." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 89, no. 12, 15 June 1992 (1992-06-15), pages 5562-6, XP000565692 the whole document --- -/--	1-11,13, 14,17-21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex

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Date of the actual completion of the international search	Date of mailing of the international search report
19 July 1999	30/07/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Le Flao, K

3

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INTERNATIONAL SEARCH REPORT

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PCT/US 99/01524

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 03173 A (BOEHRINGER INGELHEIM) 17 February 1994 (1994-02-17) page 1 - page 2; claims 1,2 ---	1-11,13, 14,17-21
X	US 5 320 962 A (STILES G L ET AL.) 14 June 1994 (1994-06-14) column 10, line 65 - column 11, line 30 ---	1-11,13, 14,17-21
A	SPEDDING, MICHAEL ET AL: "Developments in purine and pyrimidine receptor-based therapeutics." DRUG DEVELOPMENT RESEARCH, vol. 39, no. 3-4, 1996, pages 436-441, XP002108840 the whole document ---	1-23
P,A	HANSEN M.A. ET AL: "The distribution of single P (2x1)- receptor clusters on smooth muscle cells in relation to nerve varicosities in the rat urinary bladder." JOURNAL OF NEUROCYTOLOGY, vol. 27/7, 1998, pages 529-39, XP002108841 abstract -----	

3

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INTERNATIONAL SEARCH REPORT

...ernational application No.
PCT/US 99/01524

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-23
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-23 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
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3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/US 99/01524

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